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16. ABSTRACT

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Toluene Levels in Blood and Brain of Rats as a Function of Toluene Level in Inspired Air

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The relationship of toluene concentration in blood and brain to the concentration of toluene in inspired air has not been explicitly studied. Sixty rats were exposed by inhalation to 50, 100, 500, or 1000 ppm toluene for 3 hr. Immediately following exposure, venous blood samples and whole brains were collected and assayed for toluene levels. For several empirical reasons, the natural logarithm (log) of toluene tissue levels were predicted in a linear model from log toluene levels in air. An additional 10 rats were exposed to 550 ppm toluene for 8 hr in order to verify that the 3-hr exposure was sufficient to produce near-asymptotic levels of toluene in blood and brain. Log brain toluene concentration was significantly higher than log blood concentration by an additive constant. The ratio of brain to blood toluene level was estimated as 1.56/1. Three- and eight-hour exposure results did not differ, thus indicating that these results would hold for toluene exposures of 3 hr or greater.

INTRODUCTION

Toluene is a potentially neurotoxic substance commonly found in many modern environments in concentrations up to 400 ppm (Benignus, 1981). It is also an abused substance, in which context very high levels of exposure are encountered (Benignus, 1981). Quantitative knowledge of its conentration in blood and brain as a function of the concentration in inspired air is lacking although its rate of uptake and elimination are known (Benignus *et al.*, 1981). In order to design studies of the neurotoxicity of toluene it is desirable to know the pharmacodynamics of this substance in some detail, both in man and in laboratory animals.

Several studies of toluene exposure in man have been reported in which blood levels were measured (Veulemans and Masschelien, 1978; Astrand *et al.*, 1977; Gamberale and Hultengren, 1972). Concentrations of 50–700 ppm were used. While none of the studies were executed for the purpose of describing the relationship between inspired air concentration and blood levels, it was possible to make crude graphic estimates of this curve (Benignus, 1981). Little quantitative detail could be determined from such inferences about these data.

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In rats, only one study of toluene level in blood as a function of toluene exposure level was found in the literature. Pryor *et al.* (1978) exposed rats for 10 min to 13,000–70,000 ppm toluene in inspired air. The exposure levels used in the above study were very high in comparison to environmental concentrations of interest. Furthermore, the duration of exposure was too short for tissue levels of toluene to have approached asymptotic values. For these reasons the above data are of little use in the design of environmental research.

Various estimates of the ratio of brain to blood toluene have been reported or can be estimated from reports: 1.26 and 2.05 in mice (Ogata, *et al.*, 1974; and Bruckner and Peterson, 1981, respectively), 1.27 and 2.50 in rats (Pyykko *et al.*, 1977; and Pryor *et al.*, 1978, respectively). These data were collected under widely varying exposure conditions in two species and are therefore difficult to compare. Furthermore, it is not clear if the exposures were sufficiently long to have reached stable values in some cases. The ratios were sometimes based upon small samples. In some instances ratios had to be inferred from graphs. When ratios were actually reported, it was not clear whether they were ratios of means or the mean of the ratios in individual subjects. These two methods of estimation produce different results and the latter is the better estimator of the blood/brain ratio (see Methods). A large-sample estimate of inhalation-exposed subjects is required to obtain a sufficiently accurate way of estimating brain toluene level when blood level is known.

In order to determine the relationship between air concentration of toluene and blood and brain concentration, the exposure time should be long enough to allow approach to asymptotic tissue values. The time required for toluene to attain 95% of asymptotic value has been estimated as about 60 min by Benignus *et al.* (1981), using a one-compartment pharmacokinetic model. Despite the good fit to the data, some indications were present that the asymptote might have been slightly underestimated. The present study was designed to (1) obtain empirical values for blood and brain toluene levels in rats as a function of toluene levels in inspired air, (2) estimate brain/blood toluene ratios, and (3) verify that estimates of time to reach asymptote (based upon a one-compartment model) were accurate.

METHODS

Subjects. Subjects were 98 male Long–Evans hooded rats (83–95 days old, mean = 86 days, SD = 3.7). Body weights were 301–454 g (mean = 378 g, SD = 38.2). All rats were kept in an animal colony, two per cage, for a minimum of 30 days before use. One group of 28 rats was used for exploratory data analysis. These data were not, however, included in the experimental data reported. Data from another group of 60 rats were used in the confirmatory curve-fitting analysis reported below. Data from the remaining 10 rats were used to test a prediction of the one-compartment model of Benignus *et al.* (1981).

Exposure and sample collection. At each of four air–toluene levels, 50, 100, 500, and 1000 ppm, 15 rats were exposed for 3 hr. A 3-hr exposure was selected to assure that enough time was allowed to produce near asymptotic levels of

toluene in blood and brain. Earlier data (Benignus *et al.*, 1981) indicated that 1 hr would be sufficient. The exposure chamber and procedure were described in detail by Benignus *et al.* (1981). Briefly, rats were exposed in individual wire cages, five at a time, in a Rochester-type chamber. Rats were inserted and removed individually from the chamber. An additional group of 10 rats was exposed to 550 ppm for 8 hr, using the same procedures.

Rats were rendered unconscious by cervical dislocation. The rat was then immediately pinned onto a dissection board and the peritoneal cavity opened. Approximately 3.5 ml of blood was drawn from the posterior vena cava with a disposable plastic syringe. Following this the brain was removed. Blood was dispensed into an all-glass 3-ml container which was filled so that no head space remained. Brains were placed into a weighed glass bottle with a Teflon-lined needle-septum top. Bottles were weighed again to determine the amount of tissue added. Both samples were immediately placed into an ice bath. Cervical dislocation occurred within about 20 sec of removal from exposure, the blood was drawn in about 85 sec, and the brain was taken in about 185 sec (Benignus *et al.*, 1981). Blood and brain samples were analyzed by gas chromatography as described in detail in Benignus *et al.* (1981).

Statistical analyses. The study proceeded in three steps: (1) exploratory data from 28 rats were analyzed, (2) results of exploratory analyses were used to choose a model for a confirmatory experiment, and (3) the actual confirmatory experiment was done and analyzed.

The brain/blood ratio was estimated by computing the ppm ratio for each subject and then computing the mean of that ratio. It can easily be shown that the distribution of such a mean of ratios is not (in general) normally distributed. Hence, Tchebechev's inequality (Kendall and Stuart, 1977, p. 90) was used to provide approximate confidence limits.

The treatment level for an earlier study of toluene uptake rates in rats (Benignus *et al.* 1981) fell within the range of the model fitted here. In the earlier study rats were exposed for up to 4 hr to 575 ppm toluene. Consequently, it was possible to test the agreement between the values for 575 ppm predicted in this study (for both blood and brain) with those predicted by the one-compartment model in the previous study. This was done separately for brain and blood levels. Since two such predictions, one for blood and one for brain, were being compared, a multivariate test, Wilks Likelihood Ratio (Rao, 1973, p. 555) was used for the significance test. The test used the one-compartment model prediction as the hypothesized value and the error variance was estimated from the current sample. Bonferroni-corrected stepdown tests were used to follow a significant overall result.

An additional test of the earlier one-compartment model was performed by exposing 10 rats to 550 ppm for 8 hr. If the 3-hr exposure produced essentially asymptotic results, then the 8-hr exposure results should fall upon the prediction lines generated by the log–log model. The techniques used in statistical analyses are discussed in Morrison (1967).

RESULTS

Exploratory Results

In the exploratory sample of 28 rats an attempt to fit linear, zero-intercept models in ppm yielded a squared correlation (R^2) of $R^2 = .88$ ($P < 10^{-4}$) for blood and $R^2 = .86$ ($P < 10^{-4}$) for brain. A zero-intercept model is required to force a zero dose to give zero tissue level when a linear model is used. Examination of residuals, however, revealed that there was extreme heterogeneity of variance, the higher exposure values having about 1000 times the variance of the lowest one. An F test for nonlinearity was not significant for blood ($P > 0.056$) nor for brain ($P > 0.91$). This test for nonlinearity, however, assumes homogeneity of variance and is therefore not valid in this instance.

Next, the natural logarithmic (log) transforms of both air concentrations and tissue concentrations were evaluated. These transforms solved the heterogeneity of variance and improved the fit. The models for a linear relationship between log ppm in air and log ppm in tissue yielded $R^2 = .97$ ($P < 10^{-4}$) for brain and $R^2 = .96$ ($P < 10^{-4}$) for blood. F tests for nonlinearity were not significant ($P > 0.18$ for brain and $P > 0.13$ for blood). Thus all data were analyzed as log ppm and plotted on log–log graphs. This yielded (1) linearity of relationship, (2) normal distribution of residuals, (3) homogeneity of variance, and (4) improved R^2.

Confirmatory Results

For the confirmatory sample of 60 subjects, the log–log regression functions for blood and brain across exposure levels were formally compared. First, a test of coincidence was computed, which simultaneously tested whether the slopes and intercepts of the regression functions were equal. If the test of coincidence were significant, then two stepdown tests were to be done. One was a test of equality of slopes, and the other was a test of equality of intercepts. Since blood and brain levels were repeated measures on the same subject, multivariate tests were used. Therefore all of these tests were conducted by the use of Wilks' Likelihood Ratio.

Tables 1 and 2 show the means, standard deviations, and ranges for each level of toluene exposure for blood and brain, respectively. Each mean is based on 15 observations for a 3-hr exposure. Means, minima, and maxima all increased with increasing dose. The standard deviation is relatively constant across all dose levels for both blood and brain.

Figure 1 shown plots of the regression lines and the 99.9% confidence limit for predicting blood and brain log toluene concentration as a function of log toluene concentration in air. These confidence limits were chosen since the 99% confidence limits were too narrow to plot accurately. The R^2 between air log ppm toluene and blood log ppm toluene was 0.98. The corresponding R^2 for brain log ppm toluene was also 0.98. The equations for the graphs in Fig. 1 are

$$\log X = -6.53 + 1.43 (\log Z)$$

$$\log Y = -6.36 + 1.47 (\log Z),$$

TABLE 1
MEANS, STANDARD DEVIATIONS, MINIMA, AND MAXIMA FOR BLOOD TOLUENE (log ppm) AS A FUNCTION OF AIR TOLUENE (log ppm)

Exposure level (ppm)	Mean (ppm)	Mean (log ppm)	SD (log ppm)	Minimum (log ppm)	Maximum (log ppm)
50	0.45	−0.79	0.30	−1.50	−0.27
100	0.89	−0.12	0.21	−0.46	0.15
500	10.20	2.32	0.17	2.59	3.02
1000	30.60	3.42	0.13	3.24	3.68

Note. Three-hour exposures of 15 rats per level were used.

where X = blood concentration, Y = brain concentration, and Z = air concentration, all in ppm (mg/liter for blood and mg/kg for brain). The intercepts in the functions plotted in Fig. 1 had SE = 0.144 for blood and 0.147 for brain. The slopes had SE = 0.026 for blood and 0.027 for brain.

The test of coincidence showed that the regression lines for brain and blood were significantly different ($P < 10^{-4}$). The stepdown tests for differences in slope and intercept led to the conclusion that the slopes were not different ($P > 0.19$), but that the intercepts were significantly different ($P < 10^{-4}$). A difference only in the intercepts of the log–log functions corresponds to a constant ratio in the untransformed data. The average (arithmetic mean) brain/blood ratio (in ppm) was 1.56. Since this variable is non-normally distributed, Tchebechev's inequality was used to compute approximate 95% confidence limits of 1.27 and 1.84.

The log ppm value predicted for a 575-ppm, 3-hr exposure from the single-compartment model fitted by Benignus *et al.* (1981) was 2.35 for blood and 2.89 for brain. The corresponding predictions from this study were 2.56 for blood and 3.00 for brain, for a 3-hr exposure. The values from the earlier study differed significantly from the model values in this study by an overall multivariate test ($P < 10^{-4}$). The individual tests are $P < 10^{-4}$ for blood and $P < 0.01$ for brain.

Table 3 shows the log toluene levels in blood and brain as predicted from the log–log model fitted in the present study for a 550-ppm exposure. The means and

TABLE 2
MEANS, STANDARD DEVIATIONS, MINIMA, AND MAXIMA FOR BRAIN TOLUENE (log ppm) AS A FUNCTION OF AIR TOLUENE (log ppm)

Exposure level (ppm)	Mean (ppm)	Mean (log ppm)	SD (log ppm)	Minimum (log ppm)	Maximum (log ppm)
50	0.58	−0.55	0.18	−0.82	−0.15
100	1.32	0.28	0.24	−0.10	0.64
500	20.50	3.02	0.17	2.74	3.38
1000	39.20	3.67	0.18	3.90	4.01

Note. Three-hour exposures of 15 rats per level were used.

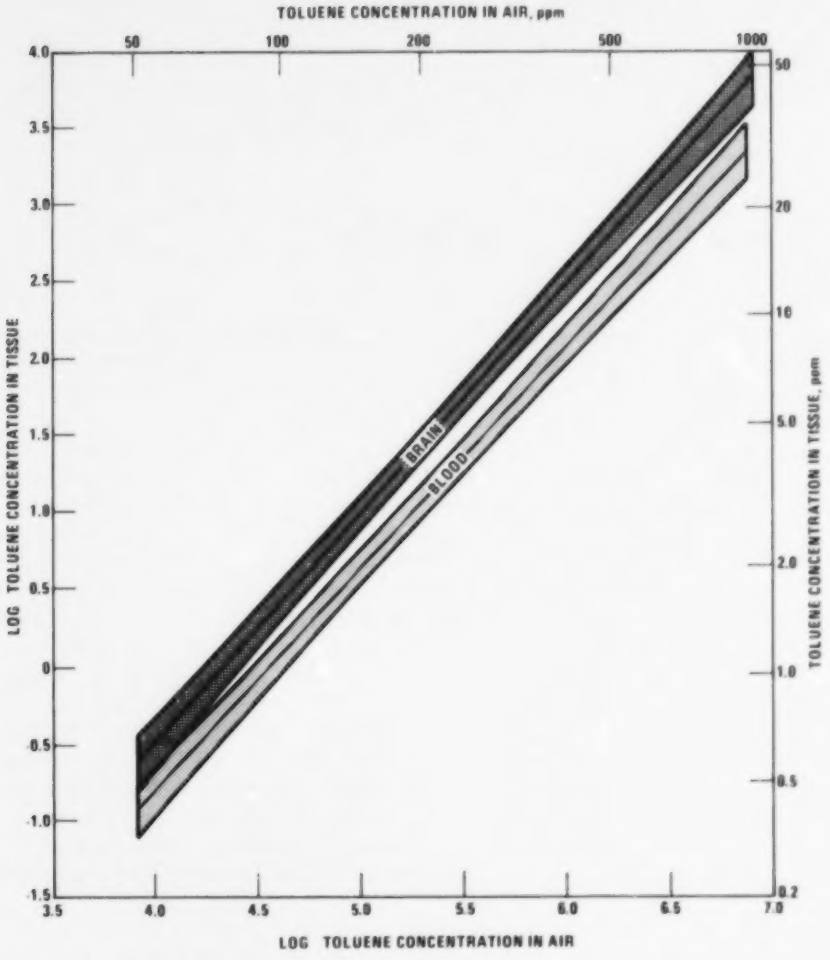

FIG. 1. Toluene levels in blood and brain as functions of toluene levels in inspired air. Graphs are lines of best fit with 99.9% confidence intervals. Values are given in log ppm (mg/l for blood and mg/kg for brain).

standard deviations of log toluene levels in blood for the 8-hr exposure animals are also given. Table 3 also reports the *t* tests to compare the predicted with the observed concentrations. The tests indicated that the toluene levels after 8-hr of exposure were comparable to the 3-hr exposure concentrations. If the two 8-hr hour points were plotted in Fig. 1, they would fall almost exactly upon the line of prediction for the 3-hr results.

DISCUSSION

One of the objectives of this work was to obtain blood and brain toluene levels as a function of toluene level in inspired air. Blood toluene levels were a linear function of air toluene levels in a log–log plot. The extremely tight confidence intervals imply that for the conditions and subjects used in this study the model is very precise.

Another objective of this study was to obtain an accurate estimate of brain/blood toluene ratio. Ratios of brain toluene to blood toluene have been reported by others, as discussed in the introduction, to range from 1.26 to 2.50. The most similar exposure regimen was used by Bruckner and Peterson (1981), who reported graphs from which a ratio of 2.05 could be estimated. These investigators used much higher exposure levels (4000 ppm) in mice which were exposed for 2 hr instead of 3 hr as done in this study. The brain/blood ratio estimated here was based on the arithmetic mean of the ratios. For the conditions in this study, this estimate of 1.56 should be quite accurate, and the confidence limits of 1.27–1.84 are likely to be conservatively large. It should be noted that the ratio for mice, estimated from Bruckner and Peterson (1981), lies outside the conservative 95% confidence limits found in this study.

The fact that the predicted toluene levels for a 575-ppm exposure were statistically different from the levels predicted by the single-compartment model of Benignus *et al.* (1981) indicates that the estimated asymptotes were slightly incorrect. However, results shown in Table 3 show that a 3-hr exposure was sufficient to produce toluene levels in both blood and brain which are very close to asymptote. It shoudl be noted, however, that (1) the discrepancy was small and (2) the one-compartment model predictions correlated about 0.91 with actual observations. The discrepancy seems to have arisen from sampling variations or from small systematic undetected errors in measurement of the exposure chamber toluene level. The estimate of time to asymptote from Benignus *et al.* (1981) was apparently correct.

SUMMARY

For rats, log concentration of toluene in blood and brain are linear functions of log concentration of toluene in air, as shown in Fig. 1. The estimated ratio of brain to blood toluene was 1.56 (1.27–1.84). These data should hold for exposure times greater than 3 hr, and possibly less.

TABLE 3

TESTS OF DIFFERENCE FROM PREDICTIONS FROM EXPERIMENT 1

	Predicted log ppm toluene	*N*	Observed mean log ppm toluene	SD	*t*	*P*
Blood	2.49	9	2.51	.39	.13	.90
Brain	2.93	10	2.92	.24	−.16	.87

Note. Values are for an 8-hr exposure at 550 ppm.

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